

## LETTER TO THE EDITOR

### **CHEK2:1100delC and Female Breast Cancer in the United States**

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Dear Sir,

Since its discovery and evaluation as a potential breast cancer susceptibility allele,<sup>1,2</sup> the *CHEK2*:1100delC mutation has been characterized in a number of settings, including several recent small series of male breast cancer patients in whom it does not appear to be associated with elevated risk.<sup>3–5</sup> Most of the studies evaluating this mutation as a female breast cancer susceptibility allele have been conducted in European populations, where the prevalence of the variant in controls ranged from 0 out of 400 controls from Spain,<sup>6</sup> 0.5% in the United Kingdom,<sup>4</sup> 1.2% in combined United Kingdom/Netherlands controls,<sup>2</sup> to 1.4% in Finland.<sup>7</sup> This variant has been detected in a considerably higher proportion (4–11%)<sup>2,7–10</sup> of family history-positive breast cancer patients (usually known to be *BRCA1/2* mutation-negative) and was estimated to be associated with an approximately 1.5- to 2-fold increased risk of female breast cancer among all such cases.<sup>2,7</sup>

The population prevalence of this mutation in the United States appears to be lower than in most European countries, although the number of subjects tested has been small: the prevalence ranged from 0.3% among 1,096 Jews and 0.4% of 569 non-Jewish controls from New York<sup>9</sup> to 0.6–0.7% among 2 other small series of 166 and 138 U.S. breast cancer controls.<sup>2,4</sup> We evaluated this mutation in 2 U.S. groups: a nested case-control study of female breast cancer from a cohort of radiologic technologists<sup>11</sup> and in 21 probands from *BRCA1/2* mutation-negative breast/ovarian cancer families.<sup>12,13</sup> The nested case-control study includes 829 prevalent cases (702 invasive and 125 *in situ* cases objectively confirmed; 2 cases confirmed by physician report only) and 859 controls without breast cancer, frequency-matched to cases on year of birth. Seventy-six cases had another cancer (including nonmelanoma skin cancer) in addition to breast cancer and 82 controls had a cancer other than of the breast. Among the breast and ovarian cancer families, the probands analyzed were affected individuals from families with at least 1 case of male breast cancer, 2 cases of ovarian cancer, or 3 cases of female breast and ovarian cancer (mean, 3.1 cases of breast cancer and 1.0 case of ovarian cancer per family) who were mutation-negative upon complete sequencing of *BRCA1/2*. No large deletions in *BRCA1* were detected in these 21 probands.

We genotyped all subjects for the *CHEK2* variant using a 5' nuclease assay on a 7900HT (Applied Biosystems, Foster City, CA). The 5  $\mu$ l reactions contained probes at 200 nM designed against the complementary strand (5'-CCCAAATCAGT AATCTAAA-3' wild-type, VIC; 5'-TGCCCAAATCATAA

TCTA-3' deletion, 6FAM) and primers at 900 nM (5'-GCAAGT-TCAACATTATTCCTTTTGT-3' and 5'-GGGTTCCACATA-AGGTTCTCATG-3') in 1X Universal Master Mix (Applied Biosystems). The reverse primer was designed to have a mismatch to both the chromosome 15 and chromosome X pseudogenes within its last 10 nucleotides. A sequence-verified positive control sample was run on each 384-well plate, and all potential positive samples were verified using dHPLC and primers described previously.<sup>14</sup> Unconditional logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for associations between *CHEK2* genotype and breast cancer risk. Multivariable logistic regression was used to evaluate the potential confounders year of birth, race, age at menarche, age at first live birth. We analyzed the data both excluding subjects with cancers other than of the breast and with multivariable regression including all subjects, but with additional adjustment for the presence of another cancer.

The mutation was present in 9 (1.1%) cases and 4 controls (0.5%) from the case-control study (Table I). Using logistic regression, the odds ratio was 2.3 (95% CI = 0.7–8;  $p = 0.16$ ), and adjustment for other risk factors did not change the estimate appreciably. The prevalences of the mutation among subjects without a cancer other than breast cancer were 0.9% among breast cancer cases and 0.4% among controls. The odds ratios were unchanged when excluding subjects with other cancers (OR = 2.3) or when adjusting for their presence (OR = 2.2). Cases were only slightly more likely to be carriers of the *CHEK2* variant if they reported a first-degree relative with breast cancer (1.2%), while similar controls were about 3 times as likely to be positive (1.6%). Mutations were more common in female breast cancer cases diagnosed before age 41 (2.5%) and those with another primary in addition to breast cancer (2.6%; one case also had melanoma, one case had nonmelanoma skin cancer; Table I). One of the 6 breast cancer controls with a history of colon cancer was *CHEK2*:

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TABLE 1 – *CHEK2*:1100delC MUTATION RESULTS

Subject group	Breast cancer cases		Controls	
	Number of positive/Number tested	% positive (95% CI)	Number of positive/number tested	% positive (95% CI)
All subjects	9/829	1.1 (0.3–1.6)	4/859	0.5 (0.01–0.9)
Excluding subjects with a different cancer <sup>1</sup>	7/753	0.9 (0.2–1.6)	3/777	0.4 (0–0.8)
Invasive breast cancer	7/702	1.0 (0.3–1.7)		
<i>In situ</i> breast cancer	2/125	1.6 (0–3.8)		
Diagnosed before age 41	4/163	2.5 (0.08–4.8)		
Family history of breast cancer				
First-degree relative (FDR)	2/161	1.2 (0–3.0)	2/129	1.6 (0–3.7)
Second-degree relative (no FDR)	2/201	1.0 (0–2.4)	1/158	0.6 (0–1.9)

<sup>1</sup>Excluding all cases with another cancer in addition to breast cancer (including nonmelanoma skin cancer) and controls with a cancer other than breast cancer.

1100delC-positive but no other mutation-positive controls had a cancer diagnosis.

In the family study, 2 of 21 (9.5%) probands were positive (1 proband had breast, ovarian and thyroid primaries), and 3 of 3 sisters with breast cancer (diagnosed at ages 45, 45 and 59) and with DNA available were also positive, although a daughter with osteosarcoma tested negative. Two female mutation carriers in the families were over age 50 and free of cancer, and the deceased parents of one of the probands had colorectal and stomach cancer.

Our results, using a population-based series of cases, although not statistically significant, are consistent with prior European studies suggesting an approximately 2-fold increased risk of breast cancer among *CHEK2*:1100delC mutation carriers. The prevalence was considerably higher among probands in the *BRCA1/2* mutation-negative multiple-case families (10%) and was carried by all known female relatives with breast cancer in the mutation-positive families, although it was not present in a daughter of one of the carriers who had osteosarcoma. The *CHEK2*:1100delC mutation was initially identified in a Li-Fraumeni syndrome family,<sup>1</sup> but the association between the 2 has not generally been verified in later studies.<sup>15</sup> The fact that this subject with osteosarcoma was mutation-negative further underscores the tenuous link between Li-Fraumeni syndrome and the *CHEK2* gene. One proband had ovarian and thyroid cancer primaries in addition to breast cancer, but none of the 5 controls from the breast cancer case-control series with ovarian or thyroid cancer was positive, nor were the 6 breast

cancer cases from the case-control series who also had ovarian, colon, or thyroid cancer.

The frequency of the *CHEK2*:1100delC mutation in the control series from the United States (0.5%; 95% CI = 0.01–0.9) appears to be somewhat lower than in parts of Europe. Although the results for female breast cancer risk are quite consistent across several studies, the very low frequency makes it difficult to demonstrate this association more convincingly in a single study. A unique aspect of this cohort is its exposure to fractionated low-dose ionizing radiation,<sup>11</sup> but the low frequency of the *CHEK2* mutation precluded our analysis of potential gene-environment interactions. Using an alpha (false positive rate) of 0.05 and a control mutation prevalence of 0.5%, more than 6,700 cases and 6,700 controls would be required to have 90% power to detect an odds ratio of 2.0 for this mutation. Even if convincingly proven in very large studies, the attributable fraction of all breast cancer (both sporadic and familial) due to the mutation is only approximately 0.6% because of its low frequency.

In summary, the *CHEK2*:1100delC mutation appears to be a rare allele that approximately doubles a carrier's risk of female breast cancer in the population setting, which may account for the familial aggregation of breast cancer in a small fraction of *BRCA1/2* mutation-negative families.

*Yours sincerely,*

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